Strategies for the Control of *Cryptosporidium parvum* Infection in Calves[1,2]

J. A. HARP and J. P. GOFF
USDA, Agricultural Research Service,
National Animal Disease Center,
Metabolic Diseases and Immunology Research Unit,
Ames, IA 50010-0070

ABSTRACT

Cryptosporidium parvum is a protozoan parasite that is now recognized as one of the leading causes of diarrhea in young calves. To date, there are no drugs or preventive measures available for the control of this disease. We have developed an oral vaccine that, when given to calves at birth, protects against experimental challenge with *C. parvum*. However, when field tested on a large dairy operation with heavy endemic *C. parvum* infection, the vaccine failed to provide protection. The difference in these results is most likely due to uncontrolled early (probably within hours of birth) exposure to *C. parvum* on the farm versus controlled exposure at 1 wk of age in the experimental trials. The successful control of *C. parvum* in the field may require vaccines that generate a rapid (within the first few days of life) cell-mediated immune response in the calf. Successful use of such a vaccine will also require improved hygiene and management practices to minimize the exposure of calves to *C. parvum* in the initial days of life, thus allowing time for protective immune responses to be generated. Careful attention to hygiene in the management of sick calves is also critical to minimize the spread of the parasite to other animals.
(**Key words**: *Cryptosporidium parvum*, calves, diarrhea)

INTRODUCTION

Cryptosporidium parvum is an intestinal protozoan parasite that causes enteric infection and diarrhea in many species of mammals (7, 16). *Cryptosporidium parvum* infection in calves has become a major economic concern for producers. In a nationwide survey of 7369 preweaning dairy calves on 1103 farms, *C. parvum* was found in 22.4% of the calves. At least 1 calf tested positive on 59.1% of the farms tested. The frequency of infection was highest in calves 1 to 3 wk of age. Forty-eight percent of the calves in this age group tested positive for *C. parvum* (8). Recently, much attention has been directed to *C. parvum* as a cause of human disease because, although *C. parvum* infection in humans usually results in self-limiting diarrhea, in AIDS (acquired immunodeficiency syndrome) patients and other immunocompromised persons, diarrhea may be chronic and life-threatening (16). Several large waterborne outbreaks of cryptosporidiosis affecting humans have occurred in recent years; the most heavily publicized of these was the 1993 Milwaukee, Wisconsin outbreak in which over 400,000 people were reported to have been infected (14). Although no source of contamination was positively identified in this and other waterborne outbreaks, contamination of watersheds by agricultural activities, such as cattle operations, have been suggested as a source (20). Thus, there is a great need to identify a means of controlling *C. parvum* infection in calves, not only to reduce the economic impact on the producer, but also to alleviate environmental and public health concerns.

A major problem in controlling *C. parvum* is the lack of an effective means for preventing or treating infection. A large number of drugs have been tested for treatment of cryptosporidiosis, but none is available that has proved to be consistently effective in a controlled trial (7, 16). Most of the drug studies have been done with AIDS patients. Those studies generally have involved few patients and have lacked a control group. Reports of success are largely anecdotal, and relapse is frequent (2). There are few studies of drug therapy against cryptosporidiosis in farm animals, and most agents tested have been either ineffective or toxic (2, 15). Paromomycin has shown efficacy in a controlled study in calves, but is not currently licensed for use in food animals (5). Thus, treatment of cryptosporidiosis in calves is limited to

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[1]Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.
[2]Invited paper.

supportive therapy (i.e., electrolytes to maintain fluid balance during bouts of diarrhea).

In this paper, we discuss the prospects for prevention of cryptosporidiosis in calves through passive or active immunization. In addition, we present strategies for controlling infection through improved hygiene and a reduction of transmission on the farm.

DISCUSSION

Passive Immunization of Calves Against *C. parvum* Infection

Passive immunity results when an immune factor is generated in one individual and transferred to another to provide protection against disease. The most common example of this is transfer of maternal antibodies in colostrum and milk to provide protection to newborns. In the bovine, intestinal pathogens such as *Escherichia coli* and rotavirus can be successfully controlled by vaccinating the cow weeks or months before calving, which increases protective antibody contents in colostrum and milk. Several studies have examined the ability of colostral antibodies to provide protection against *C. parvum*. In one study, calves receiving colostrum from hyperimmunized cows shed oocysts and had diarrhea for fewer days than did control calves (4). However, none of the calves was completely protected from infection. In that study, cows were rigorously immunized directly in the mammary gland, using oil adjuvants, a procedure that would not be suitable in a production environment. In a study conducted at the National Animal Disease Center (Ames, IA), Harp et al. (12) immunized a group of pregnant cows with a series of intramuscular injections of *C. parvum* oocysts (first suspended in oil adjuvant and subsequently in saline). Following parturition, colostrum was collected from these cows and measured for antibody to *C. parvum*. Those samples with the highest response were pooled and used in the experiment described herein.

Six calves received pooled colostrum that contained high concentrations of *C. parvum* antibody; administration of colostrum began at birth and continued six times a day for 7 d. Calves were then fed milk replacer six times a day for the next 7 d. A control group of 6 calves received one feeding of control colostrum (taken from a nonimmunized cow) at birth and then milk replacer six times a day for 14 d. At the second feeding (d 1), all calves in both groups were orally inoculated with *C. parvum* oocysts. Diarrhea and oocyst shedding were monitored daily for all calves. There were no differences in the onset or duration of either oocyst shedding or diarrhea between the two groups. Thus, no benefit was derived despite the intense dosing (six times per day for 7 d) with colostrum containing antibody to *C. parvum*.

A probable reason for the inability of colostral antibody to protect calves from infection is that *C. parvum* is an intracellular parasite. The parasite invades and carries out its life cycle primarily within intestinal epithelial cells lining the gut of the calf and is, therefore, largely protected from the effects of antibody. Studies of laboratory animals and of calves indicate that protection against *C. parvum* requires the induction of cell-mediated immune responses (3, 22, 23, 24). As suggested by the name, cell-mediated immunity requires cells, specifically lymphocytes, to carry out protective effects. Although viable lymphocytes are present in milk and have been shown to survive for a time in the neonate following ingestion (13, 18), the ability of these cells to protect the neonate against infectious diseases is unclear. Thus, there is no obvious way of generating cell-mediated immunity to *C. parvum* in the pregnant cow such that this protection would be transferred to the calf in colostrum and milk. Another way of protecting against *C. parvum* infection would be to generate an active immune response directly in the calf.

Active Immunization of Calves Against *C. parvum* Infection

Active immunity is that response generated by the immune system following exposure to a vaccine or microorganism. Both antibodies and cell-mediated immunity can be generated by active immunization. Thus, it might be possible to induce cell-mediated immunity to protect calves from cryptosporidiosis by vaccinating calves as soon as possible after birth with a killed or attenuated preparation of *C. parvum*. Because infection with the parasite is confined to the gastrointestinal tract, it seems logical to deliver the vaccine in a way that would most quickly stimulate this part of the immune system. Therefore, we (10) developed an oral vaccine made from killed *C. parvum* oocysts and tested its ability to protect calves from oocyst shedding and diarrhea caused by *C. parvum*. We found that this preparation provided specific protection against experimental challenge with *C. parvum* when given to calves within several hours of birth.

Vaccine for these studies was prepared by lyophilizing purified *C. parvum* oocysts suspended in saline. Lyophilization has been demonstrated to kill *C. parvum* (19), and the dried preparations can be stored at room temperature (18 to 30°C) for several months. The study was conducted using 19 Jersey

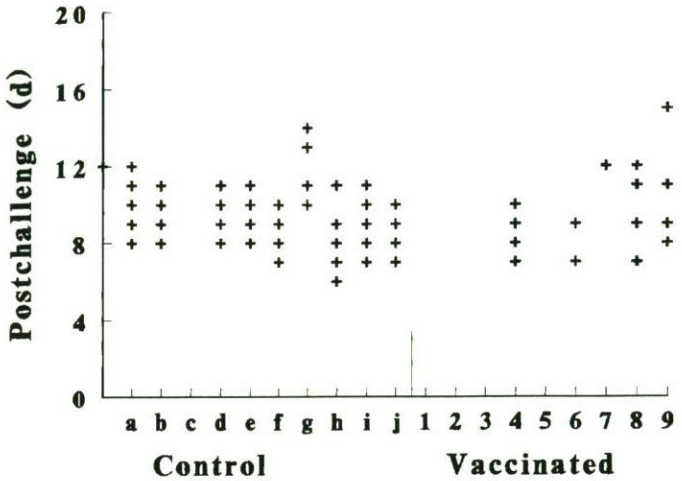

Figure 1. The onset and duration of diarrhea in calves experimentally infected at 1 wk of age with viable *Cryptosporidium parvum*. Vaccinated calves received a dose of killed *C. parvum* vaccine shortly after birth. Control calves were similarly treated but received no vaccine. Each point on the graph represents a day after challenge that individual calves were scored as positive for diarrhea. From Harp and Goff (10).

calves of both sexes. Calves were taken from their mothers at birth; 9 calves were given an oral inoculation of lyophilized *C. parvum*, and 10 calves were unvaccinated controls. All calves were given one feeding of normal bovine colostrum and thereafter were fed twice daily with commercial milk replacer. Calves were housed in an isolation barn in individual stalls for the duration of the study. All calves were observed twice daily for diarrhea and other clinical signs, and a daily sample of feces was collected from the rectum of each calf. These samples were examined for the presence of *C. parvum* oocysts by the carbol-fuchsin staining technique. At 1 wk of age, all calves were experimentally challenged by oral inoculation with live *C. parvum* oocysts. Observation continued until 4 wk of age, which was sufficient time for diarrhea and oocyst shedding to appear, run their course, and resolve.

Figure 1 shows the onset and duration of diarrhea in vaccinated and control calves that were exposed to *C. parvum*. The mean day of onset of diarrhea was d 8 for both control and vaccinated calves. The mean duration of diarrhea was 4 d for control calves and 1.7 d for vaccinated calves ($P = 0.013$). Nine of 10 control calves had diarrhea, but only 5 of 9 vaccinated calves had diarrhea. Figure 2 shows the onset and duration of oocyst shedding in these same calves. The mean day of onset of oocyst shedding was d 7 for control calves and d 9 for vaccinated calves. The mean duration of oocyst shedding was 5.3 d for control calves and 2 d for vaccinated calves ($P = 0.0004$). All 10 control calves shed oocysts, but only 6 of 9 vaccinated calves shed oocysts.

Thus, in this study, the duration of diarrhea and oocyst shedding was significantly reduced when calves were vaccinated by oral exposure to lyophilized *C. parvum* during the first few hours of life. Eight of 10 control calves had a typical course of diarrhea after experimental challenge with *C. parvum*. Diarrhea commenced about 1 wk after exposure and continued uninterrupted for 3 to 6 d. In contrast, in the vaccinated group, 4 of 9 calves had no diarrhea, and 4 others had intermittent diarrhea for 1 to 4 d. Only 1 of 9 vaccinated calves had a typical course of diarrhea (Figure 1). Similarly, oocyst shedding was reduced in vaccinates compared with controls. All of the control calves shed oocysts, beginning ~1 wk after exposure and continuing for 3 to 7 d. In contrast, 3 of 9 vaccinated calves shed no detectable oocysts, and 5 of the 9 had an abbreviated or interrupted course of shedding (Figure 2). Only 1 calf had a typical course of oocyst shedding, and that calf was the same one that had a typical course of diarrhea. This result suggests that the vaccine was ineffective in this particular calf. Perhaps the variability inherent in an outbred species might result in failure to protect some individuals.

Vaccinated calves appeared to be shedding numbers of oocysts on a daily basis that were similar to those shed by control calves (data not shown). However, vaccinated calves shed oocysts for signifi-

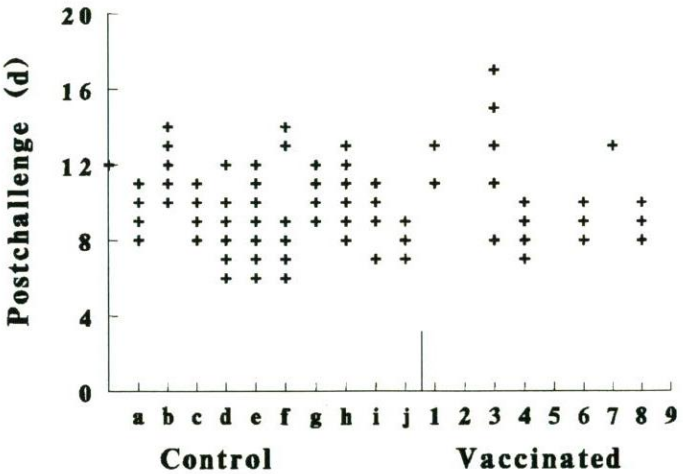

Figure 2. The onset and duration of oocyst shedding in calves experimentally infected at 1 wk of age with viable *Cryptosporidium parvum*. Vaccinated calves received a dose of killed *C. parvum* vaccine shortly after birth. Control calves were similarly treated but received no vaccine. Each point on the graph represents a day after challenge when calves were scored positive for oocyst shedding. From Harp and Goff (10).

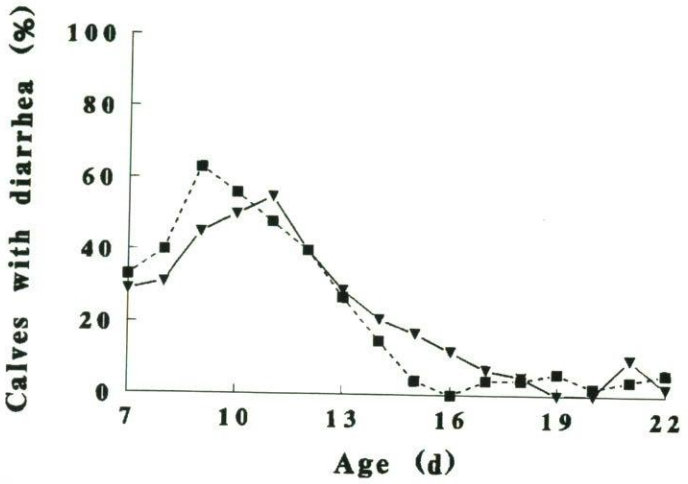

Figure 3. The percentage of calves having diarrhea between 7 and 22 d of age. Forty-two calves were enrolled in the vaccine group (▼), and 48 were in the control group (■). From Harp et al. (11).

cantly fewer days than did control calves (2 d vs. 5.3 d). Thus, vaccination may result in a reduction in the total numbers of oocysts shed into the environment, which could be an important factor in reducing the load of pathogens present and could help to break the cycle of on-farm transmission of cryptosporidiosis. Additionally, reduction of oocyst shedding may have significant impact on controlling contamination of the environment and potential waterborne spread of disease.

Because of the success of this vaccine in experimental trials, Harp et al. (11) tested the efficacy of the vaccine in a field situation. A large (~1500 cow) dairy in California with an endemic cryptosporidiosis problem was identified for study. Calves were randomly assigned to either vaccine or control groups at birth. Calves in the vaccine group (n = 42) received an oral dose of *C. parvum* vaccine as just described, and control calves (n = 48) were not treated. Beginning at 7 d of age and every day thereafter for 16 d, calves were evaluated for diarrhea. Also, beginning on d 7 and then on alternate days for 16 d, fecal samples were collected from the rectum of each calf. Fecal smears were stained with carbol fuchsin and then were examined for *C. parvum* oocysts.

Figure 3 shows the daily percentage of calves having diarrhea in the vaccinated and control groups. These percentages were maximal for both groups at 9 to 11 d of age when about 60% of the calves had diarrhea. No significant differences existed between the two groups on any of the days observed. Figure 4 shows the percentage of calves in the vaccinated and control groups that shed oocysts. These percentages were maximal at 11 to 13 d of age when about 60% of the calves shed oocysts. As with diarrhea, differences between the two groups in the percentage of calves shedding oocysts were not significant on any day tested.

These results confirm that cryptosporidiosis was endemic on this farm. During the period of observation, >90% of the calves shed oocysts for at least 1 d, and nearly all calves had diarrhea at least 1 d. Thus, there was enough *C. parvum* in the environment to infect nearly all of the calves during the study. Importantly, there were no differences between the vaccinated and control groups in either diarrhea or oocyst shedding (Figures 3 and 4). This result is in contrast to the experimental trial conducted in which both diarrhea and oocyst shedding were significantly lower in the vaccinated calves than in the control calves.

The difference in results may be due to differences in the timing of exposure to virulent *C. parvum*. In the field trial, exposure to *C. parvum* may have occurred during the initial hours or days of life because of the high level of *C. parvum* contamination on this farm. In contrast, during the experimental trials, calves were held in isolation and were not exposed to *C. parvum* until they were 1 wk old. Thus, the calves in the experimental trial had time to respond to the vaccine and to generate a protective response prior to exposure to virulent parasites. In the field trial, early exposure to *C. parvum* following vaccination may not have allowed enough time for a protective response to develop. These results suggest that successful control of cryptosporidiosis in the field may require that im-

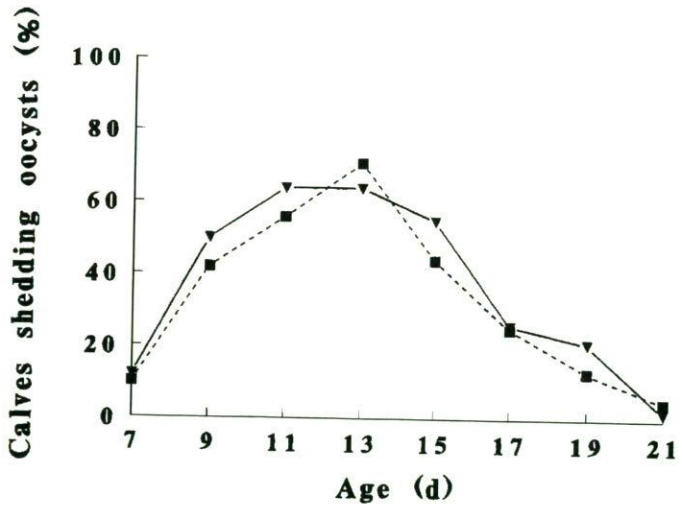

Figure 4. The percentage of calves shedding oocysts between 7 and 21 d of age Forty-two calves were enrolled in the vaccine group (▼), and 48 were in the control group (■). From Harp et al. (11).

munity in the calf be generated rapidly (within the first few days of life). We are presently working on new vaccines that will provide faster protection to calves in the field.

Prophylactic Measures to Reduce Early Exposure of Calves to *C. parvum*

In addition to improving the efficacy of vaccines, it is important for caretakers to minimize the exposure of young calves to *C. parvum*. This practice not only gives the vaccine more time to induce a protective response but, by reducing the level of environmental challenge, increases the chances of breaking the cycle of transmission. It is important to pay strict attention to hygiene in the management of sick calves. Specifically, sick calves should be housed apart from other calves, preferably in a separate building; housing sick calves in a stall next to well calves is a guaranteed way to spread disease. Caretakers should always take care of well calves before treating or handling sick calves. A separate set of boots and coveralls should be used by caretakers when caring for sick calves, and, when finished with the sick calves, clothes should be changed, and the caretaker should wash thoroughly. Although sometimes difficult in a production environment, it is important to clean stalls thoroughly before new calves are introduced. Unfortunately, one of the reasons *C. parvum* is difficult to control is its extreme resistance to disinfectants. Commercial disinfectants are generally not effective against *C. parvum* when used at recommended concentrations. Use at concentrations or exposure times that are sufficient to kill *C. parvum* would present an unacceptable hazard to humans and livestock (16). However, oocysts are susceptible to temperature extremes and desiccation. Cleaning with hot water followed by thorough drying is an effective way of killing *C. parvum*. Exposure to commercial pasteurization conditions (72°C for 15 s) killed oocysts that were suspended in water or milk (9). Freezing may also kill oocysts, depending on time and temperature. However, oocysts have been shown to survive –10°C for 1 wk (6). Thus, the best method of cleaning is to use the hottest water available and then to allow sufficient time for the area to dry thoroughly (for several days, if possible). These measures minimize spread of *C. parvum* to calves as well as to humans, especially small children or the elderly, who may be most susceptible to disease (21).

Good management practices, such as ensuring that all calves receive adequate colostrum and providing warm and dry conditions for young calves, increase the resistance of calves to other enteric pathogens. These practices are important because most calves do not die as a direct result of cryptosporidiosis, but they may die if already weakened by stress or other infections (1).

CONCLUSIONS

Research is continuing, but there are still no consistently effective drug treatments available for *C. parvum* infection in animals or humans. Treatment is limited to supportive therapy such as replacement of fluids and electrolytes. Treatment with passively transferred antibody in milk products has some palliative effect on disease symptoms in AIDS patients (17) but seems unlikely to be an economically viable option for treatment or prevention of cryptosporidiosis in calves. Active immunization of calves against *C. parvum* is in the early stages of development, and some efficacy has been shown in controlled experimental trials (10). However, the present form of vaccine does not appear to generate a response rapidly enough to protect calves in the field (11). Research is continuing in order to develop specific treatments and vaccines for cryptosporidiosis in calves. Presently, the best prescription for controlling *C. parvum* in calves is the use of sound management practices and careful attention to hygiene during the handling of infected calves.

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